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PRINCIPAL INVESTIGATOR: Dean P. Edwards, Ph.D.

CONTRACTING ORGANIZATION: University of Colorado
Health Sciences Center
Aurora, Colorado 80045-6508

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The training program is designed to graduate well-qualified and highly motivated scientists who will make a career in the breast cancer research field and who will have a strong potential for contributing new research approaches to the breast cancer problem. The students accepted into the program have already entered into different Ph.D. degree granting programs that each have their own guidelines, curriculums, and requirements. The curriculum of the Breast Cancer Training Program extends beyond that of the normal Ph.D. requirements to include didactic classroom teaching, journal clubs, seminars, workshops and mini-symposiums on relevant topics in breast cancer. Additionally, the program provides extensive one-on-one laboratory training in breast cancer research that is committed to the discovery of new fundamentals about the biology of breast cancer and its eventual treatment. The faculty who serve as research mentors have established records of successful training of Ph.D. and M.D./Ph.D. students.

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TRAINING AND KEY RESEARCH ACCOMPLISHMENTS

I Student Trainees and Training Faculty

A) Students

The table below lists the six students supported during the 2003-04 academic year on the training grant, along with their faculty thesis advisors and Ph.D. programs.

Student	Type of Student	Faculty Mentor	Ph.D. Program
Reichenberger, Kelly .	Ph.D.	Heide Ford, Ph.D.	Biochemistry
Smith, Christine	Ph.D.	Valerie Fadok, Ph.D.	Immunology
Harrell, J. Chuck	Ph.D.	Kathryn Horwitz, Ph.D.	Molecular Biology
Buser, Adam	Ph.D.	Dean Edwards, Ph.D.	Pathology
Yang, Hui	Ph.D.	Trevor Williams, Ph.D.	Molecular Biology
Poczobutt, Joanna	Ph.D.	A. Gutierrez-Hartmann, Ph.D.	Molecular Biology
*Patrick, Aaron	Ph.D.	Heide Ford, Ph.D.	Molecular Biology

* Aaron Patrick took Kelly Reichenberger's place on the grant in the Spring quarter 2004.

B) Training faculty.

During the past year, there have been no changes in the training faculty. The table below is a list of current training faculty.

DOD Breast Cancer Training Program, Faculty Members

Training Faculty	Dept.
Anderson, Steve, Ph.D.	Pathology
Bradford, Andrew, Ph.D.	OB/GYN
Edwards, Dean, Ph.D.	Pathology
Elias, Anthony, M.D.	Medicine
Fadok, Valerie, Ph.D./D.V.M.	NJC (Immun.)
Finlayson, Tina, M.D.	Surgery
Ford, Heide	OB/GYN
Gutierrez-Hartmann, Arthur, M.D.	Medicine/Endocrinology
Horwitz, Kate, Ph.D.	Medicine/Endocrinology
Kraft, Andrew, M.D.	Medicine
Langan, Thomas, Ph.D.	Pharmacology
Lu, Johnny, Ph.D.	AMC
McNiece, Ian, Ph.D.	Medicine
Neville, Margaret, Ph.D.	Physiology
Nordeen, Steve, Ph.D.	Pathology
Rabinovitch, Rachel, M.D.	Medicine
Schedin, Pepper, Ph.D.	AMC
Sclafani, Robert, Ph.D.	Biochemistry

Strange, Robert, Ph.D.	AMC
Westerlind, Kim, Ph.D.	AMC
Williams, Trevor	Craniofacial Biology

II Training Program Activities

A) Seminar Series. Students participated in a seminar series that was organized jointly with the UC Cancer Center Program on Hormone Related Malignancies. This is held weekly (Wednesdays at 11:00 AM) throughout the academic year and includes seminars on all topics related to endocrine and hormone regulated tumors. Students are encouraged to attend all seminars, but were required to attend the breast cancer or breast cancer related seminars. The seminars are presented by outside invited scientists as well as faculty from our training program. In addition, students each gave a 30 min seminar in May 2004 to update the group on the progress of their research projects. The DOD training grant supported two outside speakers, Dr. Dorraya El-Ashray and Dr. Stephanie Oesterreich. A schedule of the seminars is included in Appendix materials.

B) Annual Mini-course: All students and selected training faculty participated in annual mini-course organized by the Molecular Biology Program entitled: "Gene Eclipse: Blocking Gene Expression Using Small RNAs. The mini-course was presented on April 19-20, 2004, and was a two-day series of lectures and research seminars from local faculty and invited national/international experts in the emerging field of small RNA interference as a tool for specific gene silencing. The syllabus of the mini-course is provided in the Appendix.

C) Retreat on Mammary Gland Development, Function and Neoplasia. . All students and selected training faculty participated in an off-campus annual scientific retreat on mammary gland biology and breast cancer. The retreat was organized as a mixture of seminars by local faculty, invited outside scientists, and poster presentations by students and fellows. The DOD breast cancer training grant supported one of the invited speakers, Dr. Jeff Pollard (Yale University). The schedule of the retreat is included in Appended materials.

D) Didactic mini-course: " Reproductive Endocrinology". A 1-credit hr graduate course (Physiology 7840) was given by several of the training faculty for the students on the DOD breast cancer training grant and other interested graduate students. The class was held weekly for 2hrs during the Summer quarter, 2003, and included lectures on normal mammary development and involution, endocrine regulation of normal breast and breast cancer, and general topics of hormone action and physiology. (The course syllabus is included in the Appendix.)

III Outcomes resulting from training grant award.

The accomplishments of student trainees in terms of publications, participation and presentations at national/international meetings, and degrees awarded are listed below. The meetings listed were supported in part by the DOD training grant award. Brief descriptions of student research over the last year are included in Appendix Materials.

Student supported in prior years of the DOD grant:

Student: Suzanne Wardell

Degree Awarded: Ph.D. in Molecular Biology, May 2004. Thesis title: "Jun dimerization protein 2 (JDP-2) coactivates the progesterone receptor N-domain by a novel allosteric mechanism." (Mentor: Dr. Dean Edwards)

Post-Doctoral Position: Duke University Medical Center, Department of Pharmacology and Cancer Biology, July, 2004.

Students supported in 2003-2004:

Student: Adam Buser
Adam Buser is a third-year student in the Pathology Program. He successfully passed his comprehensive examination in February 2004, and was advanced to Ph.D. candidacy.

Attendance at Meetings:

Keystone Conference on Nuclear Steroids: Steroid Sisters. Keystone, CO. February 28 – March 4, 2004.

Participation in Scientific Conferences:

Poster presentation: "Mouse progesterone receptor and β isoforms exhibit distinct activities *in vitro* comparable to human receptors." Gordon Conference on Mammary Gland Biology. Barga, Italy. May 2004.

Publications: None yet.

Student: Hui Yang:
Hui Yang is a third-year Molecular Biology student. She successfully passed her comprehensive examination in 2004 and has advanced to Ph.D. candidacy.

Pre-Doctoral Fellowship Grant Application: Hui Yang submitted a DOD pre-doctoral fellowship grant application on May 13, 2004, entitled: "The role of AP-2beta in breast cancer."

Publications: None

Presentations at Meetings: None.

Student : J. Chuck Harrell
Chuck Harrell is a 2nd year Molecular Biology student. He has just started his research project and is very early in his training.

Publications: None yet.

Attendance at Meetings:

Keystone Symposia on Nuclear Receptors. Keystone, CO. February 28-March 4, 2004.

Student: Christine Smith:
Christine Smith is a 4th year Immunology student. She has successfully passed her comprehensive examination and has advanced to Ph.D. candidacy.

Publications: None

Attendance at Meetings:

Gordon Research Conference: Clearance of Apoptotic Cells, New London, Connecticut, August 3-8, 2003.

Student: Joanna Poczobutt
Joanna Poczobutt is a 2nd year Molecular Biology student. She has just started her research project and is early in her graduate training.

Publications: None yet.

Attendance at Meetings: None.

Student: Kelly Reichenberger
Kelly Reichenberger is a third-year graduate student in the Biochemistry Program. She completely and successfully passed the comprehensive examination in 2004.

Degree Awarded: Masters Degree in Biochemistry, May 2004. Kelly elected to receive a Masters Degree for her work thus far, and elected not to proceed to the Ph.D. degree.

Student: Aaron Patrick
Aaron Patrick is a 2nd year Molecular Biology student. He has just started his research project and is early in his training.

Publications: None yet.

Attendance at Meetings:

Workshop on Homeobox Genes and Tissue Remodeling: Focus on Mammary Gland Workshop. Bethesda, MD. March 2004.

APPENDIX

SEMINAR SERIES

RETREAT

COURSE OUTLINE

PROGRESS OF RESEARCH OF TRAINEES

University of Colorado Cancer Center

Joint Program in Hormone Related Malignancies & Division of Endocrinology, Metabolism & Diabetes

RESEARCH CONFERENCE: 2003 Fall Schedule

Room 623 Biomedical Research Building
Wednesdays, 11:00 AM

DATE	SPEAKER	TITLE
September 10	**Russell Marians, Ph.D.** Postdoctoral Fellow Physiology and Biophysics UCHSC	**The TSH Receptor: It's Not Just for Thyroid Anymore**
September 17	**Adrian Lee, Ph.D.** Assistant Professor Breast Center Baylor College of Medicine	**Hormonal regulation of IGF signaling in normal mammary gland development and breast cancer progression** **Sponsored by the Program in Reproductive Sciences**
September 24	**Shereen Ezzat, M.D.** Professor of Medicine Head, Endocrine Oncology University of Toronto/Mount Sinai Hospital, Toronto Canada	**FGFR4 as a Molecular Switch in Cancer and Metabolism**
October 1	**Russell R. Broaddus, M.D., Ph.D.** Assistant Professor of Pathology, Department of Pathology University of Texas M.D. Anderson Cancer Center, Houston, Texas	**Molecular Pathogenesis of Endometrial Cancer**
October 8	**Jorge Plutzky, M.D.** Director, The Vascular Disease Prevention Program Cardiovascular Division Brigham & Women's Hospital/Harvard Medical School, Boston MA	**Exogenous and Endogenous Forms of PPAR Activation**
October 15	**Clifford J. Rosen, M.D.** Chief of Medicine Maine Center for Osteoporosis Research & Education St. Joseph Hospital, Bangor ME	**TBA**
October 22	**Britta Jacobsen, Ph.D.** Regular Fellow Division of Endocrinology, Metabolism & Diabetes UCHSC	**Unliganded Progesterone Receptors in Breast Cancer: The Naked Truth**

DATE	SPEAKER	TITLE
October 29	**Stuart Tobet, Ph.D.** Associate Professor Department of Biomedical Sciences Colorado State University	**Development and sexual differentiation of the hypothalamus**
November 5	**Matt Jonsen, Ph.D.** Assistant Professor Division of Endocrinology, Metabolism & Diabetes UCHSC	**The Pit-1 β-domain: A Novel, Independent, Pituitary-Specific Transcriptional Repressor Motif**
November 12	**Peggy Neville, Ph.D.** Professor and Chief Section of Basic Reproductive Sciences, Dept. Obstetrics & Gynecology and Physiology & Biophysics UCHSC	**Microarray Analysis of a Hormone-Dependent Biological Switch**
November 19	**Robert Lindsay, M.D., Ph.D.** Chief, Internal Medicine Department of Medicine Helen Hays Hospital, West Haverstraw NY	**TBA**
November 26	**THANKSGIVING HOLIDAY**	**NO CONFERENCE**
December 3	**TBA**	**TBA**
December 10	**Celia Sladek, Ph.D.** Professor Physiology and Biophysics UCHSC	**Regulation of Vasopressin Release: Neurotransmitters, Osmotic Regulation, and Gonadal Steroids**
December 17	**Janice Kerr, M.D. and Katherine Weber, M.D.** Endocrinology Fellows Division of Endocrinology, Metabolism & Diabetes UCHSC	**Wnt-10 & Thyrotropes** **Research in Progress**
December 24	**CHRISTMAS HOLIDAY**	**NO CONFERENCE**
December 31	**NEW YEAR HOLIDAY**	**NO CONFERENCE**

Joint Program in Hormone Related Malignancies & Division of Endocrinology, Metabolism & Diabetes

RESEARCH CONFERENCE: 2004 Spring Schedule

Room 623 Biomedical Research Building
Wednesdays, 11:00 AM

DATE	SPEAKER	TITLE
January 14	**Jane Reusch, MD** Associate Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**CREB in Diabetes: A Tale of Death and Dysfunction**
January 21	**Paul F. Terranova, PhD** Professor of Medicine Department of Physiology & OB/GYN University of Kansas Medical Center	**Src Tyrosine Kinase and Ovarian Function**
January 28	**Jeffrey W. Pollard, PhD** Professor of Medicine Developmental & Molecular Biology & OB/GYN & Women's Health Albert Einstein College of Medicine, Bronx, NY	**TBA**
February 4	**Wm. Troy Donahoo, MD** Assistant Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**TBA**
February 11	**Lori Sussel, PhD** Assistant Professor Barbara Davis Center UCHSC	**Transcriptional Regulation of Islet Development**
February 18	**Sander J. Robins, MD** Professor of Medicine Endocrinology, Nutrition and Diabetes Boston University School of Medicine, Boston MA	**More VA-Hit: Fibrates, Inflammations and Insulin Resistance**
February 25	**Dorraya El-Ashry, PhD** Assistant Professor Division of Hematology/Oncology University of Michigan Comprehensive Cancer Center	**Mechanisms Underlying the Generation of the ERα-Negative Phenotype in Breast Cancer**

DATE	SPEAKER	TITLE
March 3	**Peter L. Jones, PhD** Assistant Professor Department of Pediatrics & Cell & Developmental Biology UCHSC	**Role of Homeobox Genes in Blood Vessel Development**
March 10	**Bryan R. Haugen, MD** Associate Professor of Medicine, Assistant Head Division of Endocrinology, Metabolism & Diabetes UCHSC	**Role of Retinoids and Retinoid Receptors in Endocrine Function**
March 17	**Margaret E. Wierman, MD** Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**Role of Annexin 2 in GnRH Neuronal Migration**
March 24	**Andrew P. Bradford, PhD** Assistant Professor Department of Obstetrics & Gynecology UCHSC	**Protein Kinase C in Endometrial Cancer**
March 31	**Jennifer Richer, PhD** Assistant Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**Progesterone Receptor-Regulated Genes: Beyond Microarrays**
April 7	**William M. Wood, PhD** Associate Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**Thyroid Hormone Control of Thyrotrope Cell Growth**
April 14	**Tammy E. Hedlund, PhD** Instructor Department of Pathology UCHSC	**Soy Consumption, Soy Metabolism, and the Possible Prevention of Prostate Cancer in Healthy Caucasian Men**
April 21	**Angela Trobaugh-Lotrario, MD** Fellow Department of Pediatrics UCHSC	**E2F-Dependent Control of Transcription and Erythropoiesis**
April 28	**Jim Lambert, PhD** Instructor Department of Pathology UCHSC	**Vitamin D Regulation of Prostate Derived Factor (PDF) Gene Expression in Human Prostate Cancer Cells**
May 5	**John Tentler, PhD** Assistant Professor of Medicine Division of Endocrinology, Metabolism & Diabetes UCHSC	**Transgenic Approaches to Understanding the Regulation of Pituitary Hormone Gene Expression**

DATE	SPEAKER	TITLE
May 12	**DOD Student Seminars** **Chuck Harrell, BS**	**Progesterone Receptors in Breast Cancer Metastasis: Development of an *in vivo* Model System**
	Joanna M. Poczobutt, BS	**Role of Integrin-Linked Kinase (ILK)-Induced Changes in Cellular Environment Leading to Transformation of Mammary Epithelial Cells**
May 19	**DOD Student Seminars** **Kelly Jansky Reichenberger, BS**	**Understanding *Six* Family Member Function in Breast Cancer**
	Adam C. Buser, BS	**Progesterone Inhibition of Milk Protein Gene Transcription in Mouse Primary Mammary Epithelial Cells**
May 26	**Jay D. Horton, MD** Associate Professor of Medicine Departments of Molecular Genetics & Internal Medicine University of Texas Southwestern Medical Center, Dallas TX	**TBA**
June 2	**No Conference**	**No Conference**
June 9	**Rocio Pereira, MD**	**Role of Adipocytes in Insulin Resistance and the Metabolic Syndrome**
	Victoria Catenacci, MD Fellows Division of Endocrinology, Metabolism & Diabetes UCHSC	**Patterns of Physical Activity in the Reduced Obese**

Last Updated 12/19/03 AG

PPG RETREAT
AGENDA
Radisson Stapleton Plaza
3333 Quebec St.

Thursday, January 29, 2004

5:00 p.m.	Host bar/buffet dinner – **Conference Room 9**
7:00	Introduction - Peggy Neville **Conference Room 10**
7:10	**Setting the Framework:** Temporal Analysis of Gene Expression – Peggy Neville
8:00	**Endocrine Regulation** Progesterone – Dean Edwards

Friday, January 30, 2004

7:30 a.m.	Continental breakfast – **Conference Room 10**
8:30	**Endocrine Regulation (contd)** Prolactin, Akt, Glut 1 – Steve Anderson
9:30	Tight junction regulation – Peggy Neville
10:00	Break with posters
10:30	**Cell Biology Session**
10:35 11:30	Kathryn Howell Jim McManaman
12:30 p.m.	Lunch and poster session – **Conference Room 9**
2:00 2:05 2:35 3:00	**Involution** The involution process—Jen Monks A potential role for PKCdelta--Mary Reyland The phosphotidyl serine receptor and cell clearance--Valerie Fadok,
3:30	Break
3:45	**Reports from Cores** – Jerry Schaack, Heide Ford
4:30	Other interacting scientists – Pepper Schedin, Trevor Williams
5:30	Adjourn

6:30 Banquet – LeDelice, 250 Josephine
Speaker: Jeff Pollard "Macrophages are required for development of bones, brains and mammary glands as well as promoting tumor metastasis"

Saturday, January 30, 2004

8:00 AM Continental breakfast – **Conference Room 10**

Advisory Committee – Jeff Rosen, Jeff Pollard, Ian Mather

Attendees - Peggy Neville, Kathryn Howell, Jim McManaman, Dean Edwards, Valerie Fadok, Mary Reyland, Steve Anderson, Steve Nordeen, Heide Ford, Jerry Schaack

REPRODUCTIVE ENDOCRINOLOGY
AN INTRODUCTORY COURSE

Mondays, 8:30 to 9:30 AM from June 30 through August 25, Room 4434.
Companion course: Topics in Reproductive Endocrinology, literature discussions to provide the research background for the didactic lectures. One hour per week, time to be announced.

Each of these courses can be taken for 1 hour of graduate credit as:
Topics in Physiology, Phys 7840.
An additional hour of independent study can be added for a total of 3 credits.

June 30	Introduction to peptide and steroid hormones Peggy Neville, Ph.D.
July 7	Pituitary hormones: the hypothalamic pituitary axis Andrew Bradford, Ph.D.
July 14	Thyroid hormone and thyroid disease Bryan Haugen, M.D.
July 21	Synthesis and regulation of steroid hormone secretion Doug Wolf, Ph.D.
July 28	Estrogen and progesterone and their mechanisms of action Doug Wolf, Ph.D.
August 4	Growth Hormone, Prolactin and the posterior pituitary Andrew Bradford, Ph.D.
August 11	GnRH, the pituitary gonadotrophin axis and puberty Margaret Wierman, M.D.
August 18	The endocrinology of pregnancy Peggy Neville, Ph.D.
August 25	Mechanisms of parturition Peggy Neville, Ph.D.

STUDENT RESEARCH PROGRESS

Student Name: Adam Buser

Mentor: Dean Edwards, Ph.D.

Progress Report:

The Role of Progesterone Receptor in Repression of Milk Protein Gene Transcription. Progesterone has two opposing biological actions during the structural and functional development of the pregnant mammary gland. A proliferative action stimulates ductal side branching and formation of lobuloaveoli. At the same time progesterone has the anti-differentiative effect of repressing milk protein gene expression, e.g. β-casein, until parturition. The primary hormone responsible for regulation of milk protein expression is prolactin whose effect is mediated through activating interaction of the Stat5 transcription factor with the promoters of milk protein genes. The goal of this project is to define the mechanism of this progesterone receptor (PR)-dependent inhibition of β-casein transcription *in vitro* and *in vivo*. We are currently investigating this phenomenon using a variety of biochemical methods as well as tissue culture methods. *In vitro* results indicate that progesterone inhibits β-casein expression at the level of gene transcription through a direct interaction of PR at the β-casein promoter that interferes with prolactin/Stat5 signaling. Electrophoretic mobility shift assays demonstrate that Stat5 and PR are able to co-bind on the β-casein promoter region containing PRE half-sites flanking a Stat5 response element. Additionally, PR is shown to block Stat5 signaling in cell culture experiments in which we have reconstituted the full PRL/Jak2/Stat5 pathway in Cos-1 cells using human PR. We have PR knock-out mice at our disposal and would like to study progesterone receptor's effects in a mouse model system, and so we have made several mouse PR reagents to study this. We have tested these mouse reagents (mammalian expression plasmids, adenoviruses, and baculoviruses) and shown that they behave similar to our human reagents in standard steroid receptor biochemical assays as well as in our cell reconstitution assays. We have also developed a way of culturing primary mammary epithelial cells from pregnant mice to maintain endogenous Stat5 signaling and shown infection with mouse PR adenovirus knocks-down endogenous protein levels of β-casein. We are currently looking for a possible mechanism whereby PR represses Stat signaling. One approach we are looking at is examining the effects of PIAS (Protein Inhibtors of Activated Stats) proteins on Stat5 signaling in our system. PIAS proteins are known to bind nuclear receptors and coactivate gene transcription, so it is possible that PR recruits one or more of these proteins to the DNA to disrupt Stat gene transcription.

Student Name: J. Chuck Harrell

Mentor: Kathryn Horwitz, Ph.D.

Progress Report:

Metastatic breast cancers kill 50,000 American women each year. More than two-thirds of these tumors retain estrogen (ER) and/or progesterone (PR) receptors. Despite clinical evidence that ER+/PR+ tumors metastasize, not much data has been obtained to determine the role of hormones or their receptors on this process. We are unaware of any experimental models to study breast cancer metastasis of E-dependent ER+/PR+ tumors. Therefore, we have developed a model of estrogen dependent breast cancer metastasis that will assess influence of PR on E-dependent metastasis. Clinically it has been shown that breast cancers overexpressing PR-A vs. PR-B are more likely to recur after tamoxifen treatment. By using a fluorescent cancer cells expressing ER and/or PR, this model we will be able to track metastatic cells *in vivo* and answer the question do PRs influence E-dependent metastasis?

We have been able to successfully generate the model and have observed ER+ metastasis to numerous organs in a PR negative breast cancer cell line (T47D). Interestingly, we have seen consistent lymphatic metastases using this model, a rarity noted in the literature. Current and future studies will determine the effects of PR on metastatic rate, location, and response to endocrine therapy.

Student Name: Aaron Patrick

Mentor: Heide Ford, Ph.D.

Progress Report:

Human Six1 (HSIX1) is a homeobox gene that is implicated in both normal development and tumorigenesis. Six1 knockout mice lack kidneys, have inner ear defects, and die at birth as a result of altered myogenesis (inadequate diaphragm muscle). These phenotypes are all caused by a reduction in proliferation. We have demonstrated that Six1 overexpression increases proliferation via the embryonic and germ line specific cyclin A1, can transform NIH 3T3 and MCF 12A cells, attenuates the G2 cell cycle checkpoint, and increases tumor burden in nude mice. Furthermore, we have found that HSIX1 is re-expressed in 44% of primary breast cancers and 90% of metastatic lesions. Interestingly, HSIX1 was recently shown to be a key metastatic regulator in a rhabdomyosarcoma (RMS) model possibly by upregulating ezrin which is implicated in the metastatic spread of mammary and pancreatic adenocarcinomas and osteosarcomas.

The objective of my project is to determine the structure of HSIX1 and to demonstrate how its DNA binding domain interacts with a target promoter at the atomic level. The hypothesis is that misexpression of HSIX1 upregulates a number of genes involved in cellular proliferation, as well as in additional properties related to metastasis, and that structural knowledge will allow "rational" structure based drug design in the future.

I have created GST-HSIX1 fusion constructs and I have worked out a purification scheme for HSIX1 to obtain protein at quantities and purity required for

crystallization. In the immediate future I plan on setting up crystal trays with Six1 alone and to begin screening DNA sequences.

Student Name: Joanna Poczobutt

Mentor: Arthur Gutierrez-Hartmann, Ph.D.

Progress Report:

Hypothesis: The central hypothesis of my project is that mammary epithelial cells transformed by ILK can confer the malignant phenotype to their non-transformed epithelial neighbors by changing their interactions with extracellular matrix and disrupting cell-cell interactions.
Study Design: As a model of a non-transformed mammary epithelial cell, I chose the MCF10A cell line, which is spontaneously immortalized, but nontumorigenic. I plan to generate cells that are stably transfected with green fluorescent protein (GFP) as a marker and doxycycline-inducible ILK. Their transformed character will be confirmed using soft agar colony formation assay and 3D organoid assay.
To examine if ILK-transformed cells can confer the malignant phenotype to their non-transformed neighbors, I will mix cells stably transfected with inducible ILK vector with untransfected, wild-type MCF-10A cells at different ratios, plate these cell combinations on matrigel and then induce ILK with doxycycline. I will use immunohistochemistry to assess transformation in the untransfected, unmarked MCF10A cells using the following criteria: loss of acinar organization, disruption of basal membrane and loss of cellular polarization (staining for laminin V, integrin $\alpha 6$ and integrin $\beta 1$), disruption of adherens junctions (loss of E-cadherin), acquisition of vimentin, increased phosphorylation of MAPK and AKT and nuclear localization of β-catenin. The inducible system will allow me to assess whether the ILK-transformed cells can confer the transformed phenotype upon their neighbors and to determine whether this effect is reversible.
Progress: I have constructed pBABEhygro-rtTA2s-M2 retroviral vector, which constitutively expresses a modified reverse tet-transactivator. I have also constructed the inducible pTREtight-ILK-V5 plasmid, which expresses V5-tagged ILK from doxycycline-responsive promoter. In transient transfections of MCF-10A cells, these vectors express ILK at high levels when induced with 1μg/mL doxycycline; with negligible expression in the absence of doxycycline. I have also generated cells stably transduced with pBABEhygro-rtTA2s-M2 retrovirus, which show robust expression of ILK-V5 when transiently transfected with the TRE-ILK-V5 inducible plasmid.
Directions for immediate future: Construct self-inactivating lentiviral vector for inducible expression of ILK. Generate cells stably transduced with both tet-transactivator and inducible vectors.

Student Name: Hui Yang

Mentor: Trevor Williams, Ph.D.

Progress Report:

Project 1: analysis of the role of AP-2beta in mouse mammary gland development. AP-2beta has been related to breast tumor in previous animal model study and clinical sample study, however, further studies of the role of AP-2beta in mammary gland have been hampered by the lack of suitable immunological reagents and animal models. To study the role of AP-2beta in mouse mammary gland, I have been trying to: 1) generate suitable immunological reagents to AP-2beta; 2) make conditional allele of mouse AP-2beta for generating mammary gland specific AP-2beta knockout mice. In the past year, we designed a specific peptide for making AP-2beta antibody. The peptide was generated commercially and three rabbits were immunized with the peptide. The antisera were characterized by Western blot and immunohistochemistry. Preliminary results indicated that the antibody we generated is a promising antibody for AP-2beta. More experiments will be done to characterize the antibody. At the same time, I constructed an AP-2beta targeting vector, which has been electroporated into mouse ES cells for making conditional AP-2beta allele. I am in the process of screening for ES cells with homologous recombination. After we get the positive ES cells, they will be karyotyped and injected into mouse blastocysts to make chimeric mice.

Project 2: analysis the role of AP-2gamma and AP-2alpha in mammary gland. Both AP-2alpha and AP-2gamma have been shown to be important in mammary gland proliferation, differentiation, and tumorigenesis by our lab. As part of the project, I characterized the phenotype of the mammary gland specific KO of AP-2gamma and AP-2alpha+gamma mice (cross MMTV-cre mice with conditional allele of AP-2alpha and AP-2alpha+gamma mice). These mice died early during embryogenesis, which is different from our expectation. To investigate the reason of these phenotypes, I have been characterizing the exact expression pattern of the MMTV-cre mice by crossing them with the Rosa26 reporter mice. More experiments are needed to get a definite conclusion.

Student Name: Christine Smith

Mentor: Valerie Fadok, Ph.D.

Progress Report:

Efferocytosis was originally described as a unique mechanism of uptake used for the clearance of apoptotic cells by professional phagocytes. Efferocytosis was described as unique because it is different than standard phagocytosis-type mechanisms (Fc-mediated phagocytosis) and is more like a macropinocytosis mechanism of uptake. Based upon work done with macrophages, six main features were established in defining efferocytosis. Over the past year, this project focused on using these six features in defining the mechanism of uptake used by non-professional phagocytes in the clearance of apoptotic cells. From this, a hypothesis was established that mammary epithelial cells use an efferocytosis type mechanism for the uptake of apoptotic cells.

To investigate the mechanism used by non-professional phagocytes, three *in vitro* model systems were established. The first model system was for the engulfment of apoptotic cells. For this, the mouse, mammary epithelial cell line, EpH4, was used as the phagocyte. The apoptotic targets used for these studies were murine, apoptotic, epithelial cells shed into the lumen of an involuting, mammary gland and milked from the gland 24hours post-wean. As part of establishing this model system, the apoptotic target and phagocyte were further characterized. For example, the median size of the apoptotic target was found to be 17 microns with a range of 10-25 microns. Typical of certain types of apoptosis, these apoptotic cells were also found to ladder their DNA and cleave caspase-3. The presence of opsonizing proteins that could potentially play a role in their uptake is still being investigated. Lastly, EpH4s were found to express the cell surface receptors $\alpha v\beta 3$, $\alpha v\beta 5$, PtdSer receptor, CD36, calreticulin, LRP-1, and Mer which could all potentially play a role in the uptake of apoptotic cells. The second *in vitro* model system established for this project was for Fc-mediated phagocytosis. Since mammary epithelial cells do not express Fc-receptors, retroviral transfection was used to establish an FcγRIIa-expressing, EpH4 cell line. These cells were shown to have stable expression of FcγRIIa, and are now being used for Fc-mediated phagocytosis assays. The third *in vitro* model system established was for macropinocytosis by EpH4 cells. For this system, addition of Salmonella typhirium is presently being tested. Once the last two model systems have been optimized, these two forms of uptake will be exposed to the same conditions used in defining the six features of efferocytosis.

Of the six features used to describe efferocytosis by macrophages, three of the six features were found to also be used by EpH4 in the uptake of apoptotic cells. First, it was shown that uptake of apoptotic cells by EpH4 was sensitive to amiloride in a dose dependent manner. Second, uptake of apoptotic cells by EpH4 cells was inhibited via pre-incubation with cholera toxin B. Similar to what was seen with macrophages, this was suggestive that clearance of apoptotic cells is a cholesterol mediated event. Third, the necessity of a Rac1/RhoA balance during the uptake of apoptotic cells by EpH4 cells was implicated by an enhancement in uptake in the presence of ROCK inhibitor (Calbiochem Y27632). To continue to define the mechanism of uptake of apoptotic cells by EpH4 cells, the last three features of efferocytosis need to be explored in EpH4 cells which includes the induction of membrane ruffling by apoptotic cells, the presence of a spacious phagosome around engulfed apoptotic cells, and a PtdSer receptor mediated bystander uptake. Lastly, additional inhibitors need to be used to confirm preliminary findings for a role of cholesterol and a Rac1/RhoA balance.